11-17-04

AF/1634
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In re the application of: Appeal No.

Rigby et al. Examiner: Sally S. Sakelaris

Serial Number: 10/017,445 Group Art Unit: 1634

Filed: December 14, 2001

For: Methods For Determining Organisms Not Requiring The Separation Of Fixative Or Excess Probe

BRIEF ON APPEAL

November 16, 2004

INTRODUCTION

This is an appeal from the action of the Examiner dated November 19, 2003, finally rejecting claims 1-21, all of the claims pending in this application. Claims 1-4, 9-12, 14, 16 and 18 stand rejected under 35 U.S.C. §102(b), as being anticipated by Yurov et al. (Human Genetics (1996)). Claims 2 and 5 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Yurov et al. (Human Genetics (1996)) in view of Drobniwski et al. (Journal of Clinical Microbiology, Jan. 2000). Claims 6 and 7 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Yurov et al. (Human Genetics (1996)) in view of Braissant et al. (Biochemica, 1998). Claims 2, 4, 8 and 21 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Yurov et al. (Human Genetics (1996)) in view of Bresser et al. (US 5,225,326). Claims 13, 19 and 20 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Yurov et al. (Human Genetics (1996)) in view of Ortiz et al. (Molecular and Cellular Probes, 1998). Claim 15 stands rejected under 35 U.S.C. §103(a) as being unpatentable

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APPEAL BRIEF
FOR APPELLANTS

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Methods For Determining Organisms Not Requiring The Separation Of Fixative Or Excess Probe

Serial No. 10/017,445
Filed: December 14, 2001
Group Art Unit: 1634
Appeal No.:

The attached Appeal Brief is submitted in accordance with the Notice Of Appeal mailed on April 30, 2004. A petition under 37 C.F.R. § 1.136 for an automatic 5-month extension of time is being filed herewith thereby making this Appeal Brief due on November 30, 2003. A request for an Oral Hearing, and payment of the appropriate fee, accompanies this Appeal Brief. The Office is authorized to deduct the appropriate fee, believed to be $ 340.00 from Deposit Account 02-3240 for the filing of this Appeal Brief as well as any other charges required for the filing and proper consideration of this paper.

Respectfully submitted,

Nov. 16, 2004
Date:

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Appeal Brief

over Yurov et al. (Human Genetics (1996)) in view of Iris et al. (US 6,403,309). Claims 11, 12 and 17 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Yurov et al. (Human Genetics (1996)) in view of Hyldig-Nielsen (US 2001/0010910). No claim stands allowed. A Notice of Appeal was timely filed on April 30, 2004 and with the automatic five-month extension, this Appeal Brief is being timely filed. Accordingly, please consider this Appeal Brief.

1. REAL PARTY IN INTEREST

The application has been assigned to Boston Probes, Inc., 15 DeAngelo Drive, Bedford, MA. USA. As of the filing of this Appeal Brief, Boston Probes, Inc. is wholly owned by Applera Corporation, acting through its Applied Biosystems stock group. These stand as the parties having an interest herein.

2. STATEMENT OF RELATED APPEALS/INTERFERENCES AND/OR JUDICIAL PROCEEDINGS

No related appeals, interferences and/or judicial proceedings pertaining to this, or any related patent or patent, application are known to be pending.

3. STATUS OF CLAIMS

Claims 1-21 stand pending in this application. Claims 1-21 stand rejected. All pending rejections pertaining to claims 1-21 are being appealed.

4. STATUS OF AMENDMENTS

All amendments of record appear to have been entered. The claims set forth in Section 9 (Appendix) reflects the entry of all amendments. No new amendments are offered.

5. SUMMARY OF CLAIMED SUBJECT MATTER

Independent Claim 1

In one embodiment, this invention is directed to a method for the analysis of organisms and/or cells. The method generally comprises first collecting a sample of organisms and/or cells to be analyzed. To the sample, as collected, is then added one or more fixative agents to thereby fix the organisms or cells. The sample is also treated with one or more molecular probes, under suitable hybridization conditions, such that the organisms and/or cells react with the molecular probe or probes in a way that produces organisms and/or cells that are detectably stained with detectable or independently detectable moieties. Once the organisms and/or cells have been fixed and stained, they are then determined. For purposes of this invention, a determination means the act of determining the presence, absence, number, position and/or identity of a cell or organism in the sample. (Specification at page 16, lines 2-14).

Independent Claim 21

According to the method, the organisms and/or cells to be analyzed can be collected from a growth medium used to increase the total number of organisms or cells available for analysis. When growth medium is used, the growth medium need not be completely separated from the organisms or cells of the sample. (Specification at page 16, line 27- page 17, line 5).

6. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

Claims 1-4, 9-12, 14, 16 and 18 stand rejected under 35 U.S.C. §102(b), as being anticipated by Yurov et al. (Human Genetics (1996)). Claims 2 and 5 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Yurov et al. (Human Genetics (1996)) in view of Drobniwski et al. (Journal of Clinical Microbiology, Jan. 2000). Claims 6 and 7 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Yurov et al. (Human Genetics (1996)) in view of Braissant et al. (Biochemica, 1998). Claims 2, 4, 8 and 21 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Yurov et al. (Human Genetics

(1996)) in view of Bresser et al. (US 5,225,326). Claims 13, 19 and 20 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Yurov et al. (Human Genetics (1996)) in view of Ortiz et al. (Molecular and Cellular Probes, 1998). Claim 15 stands rejected under 35 U.S.C. §103(a) as being unpatentable over Yurov et al. (Human Genetics (1996)) in view of Iris et al. (US 6,403,309). Claims 11, 12 and 17 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Yurov et al. (Human Genetics (1996)) in view of Hyldig-Nielsen (US 2001/0010910).

7. ARGUMENTS

I. Statement Of The Facts

Appellants reproduce below, for the convenience of the Board, such facts and evidence as support certain of the forthcoming arguments. Appellants note that facts and evidence are a product of the record. The record includes the specification, as filed, as well as each of the references considered during prosecution.[1] The source for each fact is noted with emphasized text indicated in bold, as appropriate. The first few "Facts" presented below are statements identified by the Examiner as support for the present rejection. As is argued below, Applellants do not agree that these statements/arguments are indeed

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1 See: *In re Wiseman*, 596 F.2d 1019, 1023, 201 U.S.P.Q. 658, 661 (C.C.P.A. 1979) where the court looks to the specification for support regarding Appellant's claim to unexpected results. Moreover, the court in *In re Schulze* looked to the specification for support of a claim to unexpected results by stating: "Nor do we find anything in the record by way of **disclosure** (emphasis added) or affidavit ..." (*In re Schulze*, 52, C.C.P.A. 1422, 1424, 346 F.2d 600, 602, 145 U.S.P.Q. 716, 718 (C.C.P.A. 1965) Furthermore, "In determining whether the invention as a whole would have been obvious under 35 U.S.C. § 103, we must first delineate the invention as a whole. In delineating the invention as a whole, we look not only to the subject matter which is literally recited in the claim in question (the ratio of values) but also to those properties of the subject matter which are inherent in the subject matter and are **disclosed in the specification** (emphasis added)." *In re Antonie*, 559 F.2d 618, 619, 195 U.S.P.Q. 6, 8 (Fed. Cir. 1977). Finally, what a reference teaches is a **question of fact**. *In re Bell*, 991 F.2d 781, 784, 26 U.S.P.Q.2d 1529, 1531 (Fed. Cir. 1993).

facts but rather believe these to be the expression of opinion not supported by the reference to which they pertain.

A. STATEMENTS MADE BY THE EXAMINER

Fact 1:

"The examiner acknowledges applicant's assertions that the reference did not anticipate their limitation in claim 1 of: "wherein the fixative agent or agents **and** (emphasis original) excess molecular probe or probes are not separated from the organisms or cells prior to making the determination..."(page 8 of response). However applicant's attention is directed to for example the bottom left of page 391 and the text as it continues onto page 392 as it teaches an embodiment of the Yurov method where the fixed cells and excess probe are viewed directly through the coverslip **before a washing step occurs**." (Emphasis added; OA dated Nov. 19, 2003 at page 4)

Fact 2:

"Applicant should note that although such embodiments teaching a washing step are taught in the reference itself and in the references incorporated by citation, embodiments teaching the method step **without washing are also taught in several embodiments taught by Yurov et al.**, as such the rejection stands as the teachings anticipate the invention as presently claimed." (Emphasis added; OA dated Nov. 19, 2003 at page 4)

Fact 3:

"The Examiner maintained her position that Yurov et al. anticipated the limitation of requiring **no separation**. It was explained that while the reference taught dehydration of the sample, the reference does not teach separation of the sample. **The step of dehydrating the sample is not considered to be**

equivalent to a step of separating. During dehydration not all of the fixative agent (i.e. that proportion that remains within the cell) is separated from the sample." (Emphasis added; Examiner's Interview Summary notes dated April 7, 2004 – presented with Advisory Action dated July 16, 2004)

Fact 4:

"As discussed in the interview that took place on April 7, 2004, applicants' arguments of record are not found to be convincing. As per the interview summary, it was explained that **Yurov et al. was interpreted by the examiner as not teaching separation of fixative within the fixed cell.**" (Emphasis added; Attachment to Advisory Action dated July 16, 2004)

B. FACTS ESTABLISHED BY YUROV ET AL.
Hum. Genet, 97: 390-398 (1996)

Fact 5:

"Metaphase spread and interphase nuclei were prepared from phytohemagglutinin-stimulated blood lymphocytes obtained from karyotypicallly normal individuals, patients with trisomy 21, and cultured lymphoblastoid and Hela cell lines by using standard techniques of colcemid treatment, hypotonic treatment, **and methanol acetic acid fixation**. Cell suspensions and preparations were stored in 80% methanol at –20°C **and were re-fixed before use**. Uncultured aminocyte cells were fixed as described by Soloviev et al. (1995). Slides with fixed sperm nuclei were supplied by Prof. M. R. Guichaoua (Hopital de Conception, Laboratoire de Biologie de la Reproduction, Marseille, France), Frozen samples of colorectal tumors were provided by Dr. M. Muleris (Institute Curie, Section de Biologie, Paris, France) and processed by standard techniques, utilizing collagenase treatment and **methanol/acetic acid fixation**. Samples of buccal smears were collected from a scraping of the inside of the cheek and fixed immediately with **methanol/acetic acid fixative**. (Emphasis added, Page 391. Col. 1, under heading entitled: "Cell Material")

Fact 6:

"In situ hybridization was performed as described in detail previously for isotopic in situ hybridization (Yurov 1984; Yurov et al. 1987). Slides with fixed cells (blood lymphocytes and aminocytes) **were treated with 0.07 N NaOH, 2x SSC for 30 s for chromosomal DNA denaturation, dehydrated in 70%, 96%, 100% ethanol solutions for 2 min each, and air-dryed**. Slides with interphase tumor cells, spermatozoa nuclei, or buccal epithelium cells **were treated in 2 N NaOH, 2 x SSC for 2-3 min without application of pronase or pepsin treatment, dehydrated in ethanol, and air dried**.

DNA probes at a concentration of 1-2 ng/μl in hybridization solution were denatured at 70°C for 5 min, place in ice, and then applied (7.5-10μl probes for a 22 x 22 mm coverslip) to each slide. For dural- [sic] and three color hybridization, DNA probes were mixed in equal proportions (5 μl each probe at a concentration of 5 ng/μl for each probe). Hybridization was usually performed at 42°C overnight, although clear hybridization signals were seen after 30-60 min of hybridization. **The slides were washed in 50% formamide, 2 x SSC at 42-45°C, three times for 2 min, and rinsed in 0.1-2 x SSC for 5 min**." (Emphasis added; Page 391, col. 2 under heading: "In situ hybridization and probe detection")

Fact 7:

"In experiments when Cy3-labeled probes were hybridized separately or mixed with Cy5, FluorX- or biotin-labeled probes, the step of microscopic control for hybridization efficiency was performed **without the detaching of coverslips and washing of slides**. Cy3-labeled probes usually have a low level of fluorescence in solution, and hybridization signals are clearly seen, through the cover-glass and hybridization mixture, by means of a 40x objective. If hybridization efficiency was not sufficient by visual inspection, it was possible to

continue hybridization for a longer time or to rehybridize the slides with a new sample of DNA probe after repeated denaturation of chromosomal DNA. (in 70% formamide, 2 x SSC at 70°C for 2 min followed by fixation of slides in 70%, 96%, 100% ethanol solutions for 2 min).

Directly fluorophorated probes could be analyzed immediately before (through the coverslip) or after the post-hybridization washing procedure, if necessary. Post-hybridization washing was performed to remove minor non-specific hybridization signals in 50% formamide, 2 x SSC at 42-45°C for 15 min, with microscopic analysis of the results and repetitive washing steps" (Emphasis added: Page 391, col. 2, last paragraph to page 392, col. 1, first paragraph under Heading entitled: "Control of hybridization efficacy and specificity").

C. FACTS ESTABLISHED BY YUROV, Yu. B. (Ref **CT**)
Bull. Exp. Biol. Med. (USSR), 97:643-647 (1984)[2]

Fact 8:

"1) by the method described in [10] – chromosome preparations were treated with 0.2 M HCl for 30 min and with 100 μg/ml of RNase in 2SSC (0.3 M NaCl and 0.03 M sodium citrate) for 1 h at 37°C. **Denaturation was carried out in 0.07M NaOH for 2 min, followed by washing off in alcohols**; hybridization was carried out in 2SSC at 65°C for 17h (the hybridization mixture, in a volume of 25 μl, contained in 0.01 μg DNA with specific radioactivity of 15 x 10^6 cpm/μg), the preparations were washed in two changes of 2SSC at 65°C, after which they were washed again in 2 changes of 2SSC at room temperature and in alcohols;" (Emphasis added: Page 644).

D. FACTS ESTABLISHED BY YUROV et al. (Ref **CS**)
Hum. Genet, 76: 157-164 (1987)[2]

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2 Referred to in the Materials & Methods Section of Yurov et al. (Human Genetics

Fact 9:

"We performed the preliminary experiments using the standardized *in-situ* hybridization procedure. In order to obtain reproducible results in population studies (see for additional details Yurov 1984; Yurov et al. 1986a) we used the following experimental conditions:

1. It is preferable to use in one series of experiments, the chromosomal preparations of different individuals of approximately the same "age" **after fixation**. We stored the slides for no longer than 3 months after preparation." (Emphasis added: page 158-159)

II. The Law Of 35 U.S.C. § 102

It is well settled that to be anticipatory, a prior art reference must teach each and every element/limitation of the claimed subject matter. M.P.E.P. § 2131. Moreover, the elements must be arranged as required by the claim. *Id.* "**The identical invention must be shown in as complete detail as is contained in the claim**" *Id.* quoting from *Richardson v. Suzuki Motor Co.,* 868 F2d 1226, 1236, 9 USPQ2d 1913, 1920 (Fed. Cir. 1989).

"No doctrine of patent law is better established than that a prior patent or other publication to be an anticipation must bear within its four corners adequate directions for the practice of the patent invalidated." *Dewey & Almy Chem. Co. v. Mimex Co.*, 124 F.2d 986, 989, 52 U.S.P.Q. 138, 142 (2d Cir. 1442). Accordingly it is clear that an examiner may not rely upon personal opinion or speculation to supplement the deficiencies of a reference and thereby maintain an otherwise improper rejection under 35 U.S.C. § 102.

III. Arguments In Rebuttal To The Rejection Under 35 U.S.C. § 102

It is respectfully submitted that the present controversy regarding the

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(1996) with respect to preparation of cells for *in-situ* hybridization analysis.

rejection under 35 U.S.C. § 102 appears to be based upon two very different understandings of the teachings of the Yurov et al. reference in view of the claimed subject matter. Since there can be only one correct view of the teachings of the reference in view of the claimed subject matter, this appeal is required to resolve this dispute and thereby move this application towards final disposition.

In the Office Action dated November 19, 2003, the Examiner reiterated her position that the Yurov et al. reference: "...teaches an embodiment of the Yurov method where the fixed cells **and** (emphasis original) excess probe are viewed directly through the coverslip **before a washing step occurs.**" (**Fact 1;** Emphasis added) The Examiner further argued that: "... embodiments teaching the method step **without washing** are also taught in several embodiments taught by Yurov et al...." (**Fact 2**; Emphasis added). It is believed that this interpretation of the Yurov et al. reference (Hum. Genet, 97: 390-398 (1996)) is incorrect. Such an argument was presented that the personal interview of April 7, 2004.

The relevant arguments made at the personal interview, can be summarized as follows:

1. Yurov et al. teaches, at page 391, col. 1 under heading "Cell material", that all cells were fixed with methanol/acetic acid fixative and then re-fixed before use. There does not appear to be any teaching of fixatives other than methanol/acetic acid in the Yurov reference. **Fact 5**
2. The process for in situ-hybridization discussed by Yurov et al. at page 391, col. 2, first paragraph under the heading: "In situ hybridization and probe detection" **applies to all samples analyzed.** This includes those described at page 391-392 bridging paragraphs under the heading: "Control of hybridization efficacy and specificity". The Examiner's apparent reliance on this specific teaching in Yurov et al. (See: **Facts 1 and 2**) to maintain the present rejection ignores the obvious fact that Yurov

et al. do not suggest by this text that the fixation or denaturing steps were omitted or altered for these samples, including use of the methods to which specific reference was made by Yurov et al. **Fact 6**

3. At page 391, col. 2, first paragraph under the heading: "In situ hybridization and probe detection", Yurov et al. specifically discloses the process of treating the cells with either of: 1) 0.07 N NaOH, 2 x SSC for 30 for chromosomal DNA denaturation; or 2) treatment with 2 N NaOH, 2 x SSC for 2-3 minutes without application of pronase or pepsin. Regardless, in each case the slides **are dehydrated with an ethanol series and air-dried**. This process, **which involves washing with a NaOH solution, ethanol dehydration, followed by air-drying**, would indisputably separate fixative from the cells and organisms and that all cells and organisms were treated by Yurov et al. in this fashion. It is also self evident that both methanol and acetic acid are volatile liquids that would, in addition to being **washed away**, evaporate during the air-drying step. **Fact 6** It cannot reasonably be questioned that evaporation would separate methanol and acetic acid from the cells or organisms.
4. The text relied upon by the Examiner in the Office Action as argument for maintaining the rejection (**Fact 7**), only describes samples wherein the wash occurring after hybridization is omitted and does not suggest or teach that the fix and denaturing steps (and the associated washing/separating steps) have been omitted.

The preceding arguments are further supported by the specific teachings of the two references cited by Yurov et al. Specifically, Yurov Yu. B. (Ref **CT**) teaches that "Denaturation was carried out in 0.07M NaOH for 2 min, followed by **washing off** in alcohols." (**Fact 8**) This is important since the Examiner has apparently taken the position that the "dehydration" step with alcohol is not the equivalent of a "separating" step (**Fact 3**) See the relevant arguments pertaining

to this position as set forth below.

Yurov et al. (Ref **CS**) describe preparing fixed slides that are stored for analysis. (**Fact 9)** This is important because it becomes clear that the fixation procedures used in Ref CS were also applied to samples used in the primary reference relied upon by the Examiner in maintaining the rejection.

In view of the specific teachings of Yurov et al., Appellants take the position that at least two "separation" steps are performed after fixation on every sample described by Yurov et al. (Hum. Genet, 97: 390-398 (1996). These are in order:

1) treating the cells with either of: 1) 0.07 N NaOH, 2 x SSC for 30 for chromosomal DNA denaturation; or 2) treatment with 2 N NaOH, 2 x SSC for 2-3 minutes without application of pronase or pepsin followed by dehydration with an ethanol series; and

2) air drying of the volatile ethanol and residual methanol/acetic acid, if any[3].

It is indisputable that these are the specific teachings of the reference and that every sample is described by Yurov et al. has been treated in this manner. For the avoidance of doubt, Appellants take the position that this is true notwithstanding the text of the Yurov et al. reference that appears at page 391, bottom right to page 392, top left (See the OA dated Nov. 19, 2003 at page 3-4) an which is relied upon by the Examiner to state and to maintain the present rejection (**Fact 1**).

Accordingly, it seems that the Examiner's position that Yurov et al. teaches an *in-situ* hybridization assay: "wherein the fixative agent or agents **and** excess molecular probe or probes are not separated from the organism or cells prior to making the determination" is not correct (**Facts 1 & 2**). Since the personal interview, the Examiner's view has "morphed" into an argument that "dehydrating the sample is not considered to be equivalent to a step of separating" (**Fact 3**) and that "... it was explained that Yurov et al. was **interpreted** by the examiner as

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3 The Examiner has presented no evidence that any fixative agent or agents remain

not teaching separation of fixative within the fixed cell". (**Fact 4**). Thus, the Examiner's change in position appears to recognize that reliance upon the text at the bottom right column of page 391 and to the top left column of page 392 of Yurov et al. is insufficient to maintain the rejection.

Appellants take the position that the Examiner has, on the record, stated that the rejection is maintained based upon a personal "interpretation" (**Fact 4**) of the teachings of the reference. In particular, the Examiner takes the position that dehydration is not a separations step (**Fact 3 & 4**). However, Yurov, Yu. B. (Bull. Exp. Biol. Med. (USSR)) contradicts this interpretation of the reference because it specifically describes "**washing off**" with alcohols (**Fact 8**). The Examiner's position therefore defies logic and has not been explained. Nevertheless, because the Examiner's interpretation of a reference is in conflict with statement made and relied upon by the author of the primary reference, it is respectfully submitted that the Examiner's articulated "interpretation" of the reference must be incorrect and therefore the present rejection should be withdrawn.

In addition to the foregoing, the Examiner has suggested that fix is not removed from inside of the cells or organisms and that this is a proper basis for maintaining the rejection. The Examiner makes no reference to the express teachings of Yurov et al. or to any authoritative source as support for these statements. Accordingly, they must be considered mere opinion/speculation. As discussed above, the law is clear that rejections under 35 U.S.C. § 102 must be based upon the teachings of the art and cannot be based upon an Examiner's speculation and/or opinion. Accordingly, it is believed that the present rejection is improper and should be withdrawn.

For the reasons stated, it is believed that rejection of claims 1-4, 9-12, 14, 16 and 18 under 35 U.S.C. §102 is improper and should be withdrawn.

IV. Statement of the Law of 35 U.S.C. § 103(a)

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inside or outside of the cells and/or organisms.

A. General Standard:

"A claimed invention is unpatentable if the differences between it and the prior art "are such that the subject matter **as a whole** (emphasis added) would have been obvious at the time the invention was made to a person of ordinary skill in the art." *In re Dembiczak*, 175 F.3d 994, 998, 50 U.S.P.Q.2d 1614, 1616 (Fed. Cir. 1999). In determining whether a claimed invention is obvious one must consider; 1) the scope and content of the prior art; 2) the level of skill in the prior art; 3) the differences between the claimed invention and the prior art; and 4) objective evidence of non-obviousness such as secondary factors. Id.

The PTO bears the burden under 35 USC § 103 to establish an unrebutted *prima facia* case of obviousness. *In re Rouffet*, 149 F.3d 1350, 1355, 47 U.S.P.Q.2d 1453, ___ (Fed. Cir. 1998). To satisfy its burden, the PTO must show some objective teaching in the prior art or that knowledge generally available in the art would lead the ordinary practitioner to combine **relevant teaching**. *In re Fine*, 837 F.2d 1071, 1074, 5 U.S.P.Q.2d 1596, 1598 (Fed. Cir. 1988). In the absence of a proper *prima facia* case of obviousness, an Applicant is entitled to a patent. *In re Rouffet*, 149 F.3d 1350, 1355, 47 U.S.P.Q.2d 1453, ___ (Fed. Cir. 1998). To overcome a claimed *prima facia* case of obviousness, an Applicant can either show that the *prima facia* case of obviousness is insufficient because it relies on incorrect factual predicates or otherwise present secondary evidence of non-obviousness. *Id.*

A proper rejection under 35 USC § 103 may not be premised upon "bald assertions" for which there is no support for or explanation of a conclusion. *Id.* An Examiner's cursory statement unaccompanied by evidence or reasoning is entirely inadequate to support a rejection. *In re Sichert*, 566 F.2d 1154, 1164, 196 U.S.P.Q. 209, 217 (C.C.P.A., 1977). A rejection based on section 103 must be based in fact that is not aided by hindsight. *In re Warner*, 54 C.C.P.A. 1628, 1635, 379 F.2d 1011, 1017, 154 U.S.P.Q. 173, 178 (C.C.P.A., 1967). The PTO may not resort to speculation, unfounded assumptions or hindsight reconstruction

to supply deficiencies in its factual basis for a rejection. *Id.* Doubts as the factual basis for a rejection must to resolved in favor of the Applicant since it is the PTO's burden to establish a *prima facia* case of obviousness. *Id.*

B. Application of Hindsight:

On the permissibility of an Examiner's use of hindsight, the law is quite clear. Recent courts have held that: "Our analysis begins in the text of section 103 quoted above, with the phrase "at the time the invention was made". For it is this phrase that guards against the "tempting but forbidden zone of hindsight,"." *In re Dembiczak*, 175 F.3d 994, 998, 50 U.S.P.Q.2d 1614, 1617 (Fed. Cir. 1999). Measuring the claimed invention against the standard established by section 103 requires the oft-difficult but critical step of casting the mind back to the time of invention, to consider the thinking of one of ordinary skill in the art, guided only be the prior art references and **the then-accepted wisdom in the field** (emphasis added)." *Id.* Close adherence to this methodology is especially important to avoid using the inventor's own teachings against the teacher. *Id.* The best defense against application of hindsight is **rigorous application** of a requirement for a showing of the teaching or motivation to combine the prior art references. *Id.* The examiner can satisfy this burden only by showing some objective teaching leading to the combination. *Id.* That showing must be **clear and particular**. *Id.* "Broad conclusory statements regarding the teaching of multiple references, standing alone, are not "evidence." *Id.* The mere fact that the prior art may be modified in a manner suggested by the Examiner does not make the modification obvious unless the prior art expressly suggested the modification. *In re Fritch*, 972 F.2d 1260, 1266, 23 U.S.P.Q.2d 1780, 1783-1784 (Fed. Cir. 1992). Moreover, when prior art references require selective combination, there must be some reason for the combination other than the hindsight gleaned from the invention itself. *Interconnect Planning Corp. v. Feil*, 774 F.2d. 1132, 1143, 227 U.S.P.Q. 543, 551 (Fed. Cir. 1985). The Examiner may not pick and choose

among isolated disclosures to deprecate the claimed invention. *In re Fritch*, 972 F.2d 1260, 1266, 23, U.S.P.Q.2d 1784, 1784 (Fed. Cir. 1992).

C. Obvious To Try Is Not The Standard Of Obviousness

"Obvious to try" is not a legitimate test of patentablility. *In re Fine,* at 1075, 5 U.S.P.Q.2d 1596,1599 (Fed. Cir. 1988). Admonition that the subject invention is "obvious to try" is generally directed to two types of error. In one case (motif one), that which would be "obvious to try" is to vary all parameters or to try each of numerous possible choices until one arrives at a successful result, where the prior art gave no indication of which parameters where critical and no directions as to which of many possible choices were likely to be successful. In the other case (motif two), that which is "obvious to try" is to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it. *In re O'Farrell*, 853 F.2d 894, 903, 57 USLW 2147, ___, 7 U.S.P.Q.2d 1673, 1681 (Fed. Cir. 1988).

D. Expectations of Success

Both suggestion and reasonable expectation of success must be found in the prior art *and not in Applicant's disclosure*. *In re Vaeck*, 947 F.2d 448, 493, 20 U.S.P.Q.2d 1438, 1442 (Fed. Cir. 1991).

V. Arguments In Rebuttal To The Rejections Under 35 U.S.C. § 103(a)

A. *The Rejections Are Cumulative*

All remaining rejections articulated in the present Office Action are made under 35 U.S.C. § 103(a) and are cumulative with respect to the rejection under 35 U.S.C. §102(b).[4] The Examiner relies upon arguments made in paragraph 1 of the Office Action with respect to the Yurov et al. (Hum. Genet 97: 390-398

4 See paragraphs 2-7 of the Office Action dated November 19, 2003

(1996)) reference, the accuracy of which Appellants dispute. Because all of these rejections necessarily rely upon an incorrect interpretation of the Yurov et al. reference with respect to the claimed subject matter, it is believed that all of the rejections under 35 U.S.C. §103(a) must properly be withdrawn.

B. The Rejection Based Upon Yurov et al. and Drobniewski et al.

(i) Statement Of The Rejection

At paragraph 2 of the Office Action dated November 19, 2003, the Examiner rejected claims 2 and 5 as being unpatentable over Yurov et al. in view of Drobniewski et al. (Journal of Clinical Microbiology (2000) 38(1): 444-447). The Examiner relies upon arguments made in paragraph 1 of the Office Action with respect to the Yurov et al. reference, the accuracy of which Appellants dispute.

Nevertheless, the Examiner appears to take the position that Yurov et al. does not teach various elements/limitations of the rejected claims but takes the position that Drobniewski et al. teach the missing element(s)/limitation(s). In particular, the Examiner asserts that Yurov et al. does not disclose the use of a growth medium consisting of broth or agar. (OA dated Nov. 19, 2003 at page 6).

(ii) The Rejection Is Defective

The rejection does not appear to clearly identify any specific motivation to combine the references. There is also no basis for expecting that an *in-situ* hybridization assay can be performed without some separation of broth or agar growth medium. Typically in an *in-situ* assay, excess reagents are washed away so that they do not interfere with the observation of the stained cells or organisms. For example, Drobniewski et al. teach that the step of a final wash (a separation step) is to performed before visualization of the sample under the microscope (i.e. the determination step; See: page 446, col. 1, lines 16-23).

In the present case, the Examiner assumes that the presence of broth or agar will not inhibit the determination of the cells and/or organisms but the

references provides no suggestion or teaching that would support this assumption/speculation on the Examiner's part. In fact, Drobniewski et al. specifically **teach away** from a no wash assay and there is certainly nothing in Yurov et al. that would address the issue. Accordingly, the rejection is defective because it merely combines elements of different references without providing any thought as to the result of the asserted combination. Indeed, it is believed that the combination of references is most definitely hindsight based and lacks proper motivation and/or expectation of success. Accordingly, Appellants believe that it should properly be withdrawn.

(iii) Argument Conclusion

For at least these reasons, it is respectfully submitted that the present rejection of claims 2 and 5 based upon the combination of Yurov et al. with Drobniewski et al. is improper under 35 U.S.C. § 103(a) and should be withdrawn.

C. The Rejection Based Upon Yurov et al. and Braissant et al.

(i) Statement Of The Rejection

At paragraph 3 of the Office Action dated November 19, 2003, the Examiner rejected claims 6 and 7 as being unpatentable over Yurov et al. in view of Braissant et al. (Biochemica (1998) 1: 10-15). The Examiner relies upon arguments made in paragraph 1 of the Office Action with respect to the Yurov et al. reference, the accuracy of which Appellants dispute.

Nevertheless, the Examiner appears to take the position that Yurov et al. does not teach various elements/limitations of the rejected claims but takes the position that Braissant et al. teach the missing element(s)/limitation(s). In particular, the Examiner takes the position that Yurov et al. does not teach the use of a blocking agent such as casein. (OA dated Nov. 19, 2003 at page 9).

(ii) The Rejection Is Defective

The rejection does not appear to clearly identify any specific motivation to combine the references. There is no basis to expect that an *in-situ* hybridization assay can be performed without some separation of the blocking agent. Typically in an *in-situ* assay, excess reagents are washed away so that they do not interfere with the observation of the stained cells or organisms. For example, Braissant et al. teach that: "After hybridization, optimal washing conditions that ensure signal specificity consisted of rinses of 30 min in 2x SSC at room temperature, 1 h in 2 x SSC at 65°C, and 1 h in 0.1x SSC at 65°C." At page 15, col. 2, lines 11-13, the reference reads under the heading "ISH protocol": "After hybridization, **our washing procedure ensures signal specificity**." At page 15, col. 2, lines 45-50 the reference reads: "After the removal of unspecific labeling in ethanol, a rapid rehydration step in water **was found to be necessary** in order to eliminate the tris precipitate from the tissue, which otherwise hampers histological observation.

Taken together these statements by Braissant et al. demonstrate that a separation step is most definitely contemplated and even necessary in all of their embodiments. Thus, the reference actually **strongly teaches away** from the very position taken by the Examiner. Accordingly, the rejection is defective because it merely combines elements of different references without providing any thought as to the result of the asserted combination. Indeed, it is believed that the combination of references is most definitely hindsight based and lacks proper motivation and/or expectation of success. Accordingly, Appellants believe that it should properly be withdrawn.

(iii) Argument Conclusion

For at least these reasons, it is respectfully submitted that the present rejection of claims 6 and 7 based upon the combination of Yurov et al. with Braissant et al. is improper under 35 U.S.C. § 103(a) and should be withdrawn.

D. The Rejection Based Upon Yurov et al. and Bresser et al.

(i) Statement Of The Rejection

At paragraph 4 of the Office Action dated November 19, 2003, the Examiner rejected claims 2, 4, 8, and 21 as being unpatentable over Yurov et al. in view of Bresser et al. (US 5,225,326). The Examiner relies upon arguments made in paragraph 1 of the Office Action with respect to the Yurov et al. reference, the accuracy of which Appellants dispute.

Nevertheless, the Examiner also appears to take the position that Yurov et al. does not teach various elements/limitations of the rejected claims but takes the position that Bresser et al. teach these elements/limitations. In particular, the Examiner takes the position that Yurov et al. do not teach a method wherein organisms, cells, or both, are collected from a growth medium that has not been completely separated from the sample nor do they teach a method wherein steps (b) and (c) (See claim 1) are performed simultaneously. The Examiner further states that Yurov et al. does not teach a method for determining organisms, cells or both, said method comprising:

a) treating a sample of fixed cells, organisms or both, that have been grown in a medium, with one or more detectable molecular probes, under suitable hybridization conditions, in a way that produces stained organisms, cells or both stained organisms and cells, and

b) determining the stained cells, organisms or both the stained organisms or cells;

wherein said assay does not require that the [growth] medium be removed or separated from the organisms, cells or both the organisms and cells (OA dated Nov. 19, 2004 at pages 11-12).

The Examiner then takes the position that Bresser et al. teach such methods. Appellants dispute the Examiner's interpretation of the references.

(ii) The Rejection Is Defective

The rejection does not appear to clearly identify any specific motivation to combine the references. Moreover, Appellants wish to point out that the reference teaches the operation of a separations step. At column 13 under the heading: "**Noise is Washed Away**" the reference reads: "Next, the unbound probe is washed away form the cells by one wash step using a solution of 0.1 x SSC (1x SSC = 0.15M NaCl and 0.015 M sodium citrate, pH 7.4) with 0.1% Triton X-100$_{TM}$. Applellants view that Bresser et al. teach a separation step is also fully supported by Examples 2-12 wherein in each case a post hybridization wash step or pelleting step is performed prior to detection of the stained cells. This conflicts with the Examiner's stated view of the reference.

As discussed previously, Yurov et al. most definitely teach that a separation step is performed after fixation. Consequently, these references alone and in combination simply do not teach that which the Examiner asserts that they do.

Taken together these statements by Bresser et al. demonstrate that a separation step is most definitely contemplated and even necessary in all of their embodiments. Thus, the reference actually **teaches away** from the very position taken by the Examiner. Accordingly, the rejection is defective because it merely combines elements of different references without providing any thought as to the result of the asserted combination. Indeed, it is believed that the combination of references is most definitely hindsight based and lacks proper motivation and/or expectation of success. Accordingly, Appellants believe that it should properly be withdrawn.

(iii) Argument Conclusion

For at least these reasons, it is respectfully submitted that the present rejection of claims 2, 4, 8 and 21 based upon the combination of Yurov et al. with Bresser et al. is improper under 35 U.S.C. § 103(a) and should be withdrawn.

E. The Rejection Based Upon Yurov et al. and Ortiz et al.

(i) Statement Of The Rejection

At paragraph 5 of the Office Action dated November 19, 2003, the Examiner rejected claims 13, 19 and 20 as being unpatentable over Yurov et al. in view of Ortiz et al. (Molecular and Cellular Probes (1998) 12: 219-226). The Examiner relies upon arguments made in paragraph 1 of the Office Action with respect to the Yurov et al. reference, the accuracy of which Appellants dispute. Nevertheless, the Examiner appears to take the position that Yurov et al. does not teach various elements/limitations of the rejected claims but takes the position that Ortiz et al. teach these elements/limitations. In particular, the Examiner takes the position that Yurov et al. does not teach a molecular probe that is self indicating. (OA dated Nov. 19, 2003 at page 15)

(ii) The Rejection Is Defective

The rejection does not appear to clearly identify any specific motivation to combine the references. Specifically, the combination of these references by the Examiner is most curious since Ortiz et al. does not teach or even suggest the application of their probes to an *in-situ* hybridization assay. Moreover, the primary reference, Yurov et al., does not teach the application of a self-indicating probe. In this respect, the two references are non-analogous art and are therefore improperly combined especially given the lack of common subject matter and/or relevant suggestion or teaching.

Accordingly, the rejection is defective because it merely combines elements of different references without providing any thought as to the result of the asserted combination. Indeed, it is believed that the combination of references is most definitely hindsight based and lacks proper motivation and/or expectation of success. Accordingly, Appellants believe that it should properly be withdrawn.

(iii) Argument Conclusion

For at least these reasons, it is respectfully submitted that the present rejection of claims 13, 19 and 20 based upon the combination of Yurov et al. with Ortiz et al. is improper under 35 U.S.C. § 103(a) and should be withdrawn.

F. The Rejection Based Upon Yurov et al. and Iris et al.

(i) Statement Of The Rejection

At paragraph 6 of the Office Action dated November 19, 2003, the Examiner rejected claim 15 as being unpatentable over Yurov et al. in view of Iris et al. (US 6,403,309). The Examiner relies upon arguments made in paragraph 1 of the Office Action with respect to the Yurov et al. reference, the accuracy of which Appellants dispute.

Nevertheless, the Examiner appears to take the position that Yurov et al. does not teach various elements/limitations of the rejected claims but takes the position that Iris et al. teach these elements/limitations. In particular, the Examiner takes the position that Yurov et al. does not teach a method of detection wherein the method comprises: e) adding a quencher labeled oligomer before the determination is made to thereby form a complex between excess molecular probe and quencher labeled oligomer. (OA dated Nov. 19, 2003 at page 17)

(ii) The Rejection Is Defective

The rejection does not appear to clearly identify any specific motivation to combine the references. Specifically, the combination of these references by the Examiner is most curious because Iris et al. does not teach or even suggest the application of their probes to an *in-situ* hybridization assay. Moreover, the primary reference, Yurov et al., does not teach the application of "anti-probes" to "mop-up" excess probe in an *in-situ* assay. In this respect, the two references are non-analogous art and are therefore improperly combined especially given the lack of common subject matter and/or relevant suggestion or teaching.

Accordingly, the rejection is defective because it merely combines

elements of different references without providing any thought as to the result of the asserted combination. Indeed, it is believed that the combination of references is most definitely hindsight based and lacks proper motivation and/or expectation of success. Accordingly, Appellants believe that it should properly be withdrawn.

(iii) Argument Conclusion

For at least these reasons, it is respectfully submitted that the present rejection of claim 5 based upon the combination of Yurov et al. with Iris et al. is improper under 35 U.S.C. § 103(a) and should be withdrawn.

G. The Rejection Based Upon Yurov et al. and Hyldig-Nielsen et al.

(i) Statement Of The Rejection

At paragraph 7 of the Office Action dated November 19, 2003, the Examiner rejected claims 11, 12 and 17 as being unpatentable over Yurov et al. in view of Hyldig-Nielsen et al. (US Published Application US 2001/0010910). The Examiner relies upon arguments made in paragraph 1 of the Office Action with respect to the Yurov et al. reference, the accuracy of which Appellants dispute. Nevertheless, the Examiner takes the position that Yurov et al. does not teach various elements/limitations of the rejected claims but that Hyldig-Nielsen et al. teach these elements/limitations. In particular, the Examiner takes the position that Yurov et al. does not teach a multiplex *in-situ* hybridization assay or an *in-situ* hybridization assay wherein one or more blocking probes are present during the operation of step (c) (See claim 1).

(ii) The Rejection Is Defective

The rejection does not appear to clearly identify any specific motivation to combine the references. Accordingly, the rejection is defective and should be withdrawn. Indeed, it is believed that the combination of references is most definitely hindsight based and should properly be withdrawn.

(iii) Argument Conclusion

For at least these reasons, it is respectfully submitted that the present rejection of claim 5 based upon the combination of Yurov et al. with Hyldig-Nielsen et al. is improper under 35 U.S.C. § 103(a) and should be withdrawn.

VII. General Note On The Examiner's Arguments Supporting All Rejections Under 35 U.S.C. § 103(a).

In all cases it seems that the Examiner's argument is that it would have been obvious to combine the references in the manner suggested, not because the reference teach or suggest such a combination, but rather merely to achieve the advantages of the claimed subject matter. At best the combination is "obvious to try" but not "obvious" by the standard required under 35 U.S.C. § 103(a). Accordingly, it is respectfully submitted that the rejection was constructed using the applicant's specification as a blueprint or roadmap. It is however, well accepted that hindsight based analysis is improper. Withdrawal of all rejections under 35 U.S.C. § 103 (a) on this basis is therefore earnestly requested.

VIII. SUMMARY

It is believed that this response addresses all rejections set forth in the Office Action dated November 19, 2003 and the application is in ready condition for allowance. In view of the foregoing arguments, Appellants respectfully request that the Board overrule the Examiner and thereby direct the Examiner to withdraw all pending rejections articulated in the Office Action dated November 19, 2003.

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Respectfully submitted
On behalf of Appellants

Nov. 16, 2004
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8. CLAIMS APPENDIX

Claims on appeal

1. (Original) A method for the analysis of organisms, cells or both organisms and cells; said method comprising:
   a) collecting a sample of organisms or cells;
   b) adding one or more fixative agents to the sample to thereby fix the organisms, cells or both;
   c) treating the sample with one or more molecular probes, under suitable hybridization conditions, such that the organisms, cells or both react with the molecular probe in a way that produces detectable or independently detectable organisms, cells or both; and
   d) determining one or more of the detectable organisms or cells in the sample; wherein the fixative agent or agents and excess molecular probe or probes are not separated from the organisms or cells prior to making the determination.

2. (Original) The method of claim 1, wherein the organisms, cells or both are collected from a growth medium.

3. (Previously Amended) The method of claim 1, wherein the organisms, cells or both are collected directly from a sample that has not been treated with a growth medium.

4. (Original[5]) The method of claim 2, wherein the growth medium is not completely separated from the sample of organisms, cells or both.

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5 In the amendment offered with Applicants response filed on July 23, 2003 this claim was identified as being amended but was not. As can be seen by comparison with the

5. (Original) The method of claim 2, wherein the growth medium is selected from the group consisting of broth and agar.

6. (Original) The method of claim 1, wherein a blocking agent is present during the operation of step (c).

7. (Original) The method of claim 6, wherein the blocking agent is casein.

8. (Original) The method of claim 1, wherein steps (b) and (c) are performed simultaneously.

9. (Original) The method of claim 1, wherein steps (b) and (c) are performed sequentially in that order.

10. (Original) The method of claim 1, wherein the molecular probe is labeled with a fluorophore.

11. (Original) The method of claim 1, wherein two or more independently detectable molecular probes are used in the method for the multiplex analysis of two or more different types of organisms or cells in the sample.

12. (Original) The method of claim 11, wherein the two or more independently detectable molecular probes are labeled with independently detectable fluorophores.

13. (Original) The method of claim 1, wherein the molecular probe is a self-indicating molecular probe selected from the group consisting of a linear beacon, a nucleic acid or PNA molecular beacon and an intercalating

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specification as filed, the text of Claim 4 remains Original.

beacon.

14. (Original) The method of claim 1, wherein the molecular probe is a detection complex.

15. (Original) The method of claim 1, further comprising:
    e) adding a quencher labeled oligomer before the determination is made to thereby form a complex between the excess molecular probe and the quencher labeled oligomer.

16. (Original) The method of claim 1, wherein the cells or organisms of the sample are determined using either a microscope, an array scanner or a flow cytometer.

17. (Original) The method of claim 1, wherein one or more blocking probes are present during the operation of step (c).

18. (Original) The method of claim 1, wherein the molecular probe is a nucleic acid probe.

19. (Original) The method of claim 1, wherein the molecular probe is a non-nucleic acid probe.

20. (Original) The method of claim 19, wherein the non-nucleic acid probe is a peptide nucleic acid probe.

21. (Previously Amended) A method for determining organisms, cells or both, said method comprising:
    a) treating a sample of fixed cells, organisms or both, that have been

grown in a medium, with one or more detectable molecular probes, under suitable hybridization conditions, in a way that produces stained organisms, cells or both stained organisms and cells; and

b) determining the stained cells, organism or both the stained organisms and cells;

wherein the medium is not removed or separated from the organisms, cells or both the organisms and cells.

9. EVIDENCE APPENDIX

Nothing is presented.

10. DECISIONS APPENDIX

Nothing is presented.